# Small-Molecule Inhibitors of the Wnt Pathway Potently Promote Cardiomyocytes From Human Embryonic Stem Cell-Derived Mesoderm.

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**Public Summary:**
Rationale: Human embryonic stem cells can form cardiomyocytes when cultured under differentiation conditions. Although the initiating step of mesoderm formation is well characterized, the subsequent steps that enrich for cardiac lineages are poorly understood and limit the yield of cardiomyocytes. Objective: Our aim was to develop a human embryonic stem cell-based high-content screening assay to discover small molecules that drive cardiogenic differentiation after mesoderm is established to improve our understanding of the biology involved. Screening of libraries of small-molecule pathway modulators was predicted to provide insight into the cellular proteins and signaling pathways that control stem cell cardiogenesis. Methods and Results: Approximately 550 known pathway modulators were screened in a high-content screening assay, with hits being called out by the appearance of a red fluorescent protein driven by the promoter of the cardiac-specific MYH6 gene. One potent small molecule was identified that inhibits transduction of the canonical Wnt response within the cell, which demonstrated that Wnt inhibition alone was sufficient to derive cardiomyocytes from human embryonic stem cell-originating mesoderm cells. Transcriptional profiling of inhibitor-treated compared with vehicle-treated samples further indicated that inhibition of Wnt does not induce other mesoderm lineages. Notably, several other Wnt inhibitors were very efficient in inducing cardiogenesis, including a molecule that prevents Wnts from being secreted by the cell, which confirmed that Wnt inhibition was the relevant biological activity. Conclusions: Pharmacological inhibition of Wnt signaling is sufficient to drive human mesoderm cells to form cardiomyocytes; this could yield novel tools for the benefit of pharmaceutical and clinical applications.

**Scientific Abstract:**
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